Estrous Cyclicity of Mice During Simulated Weightlessness

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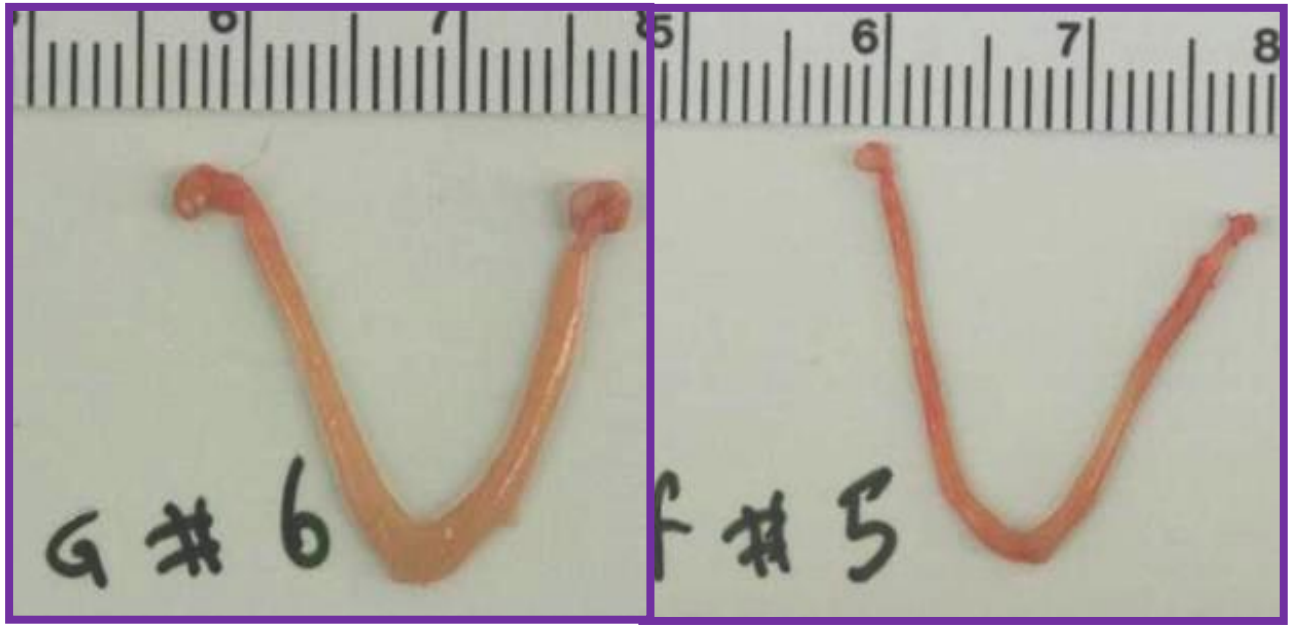
Presented at the 2017 meeting of American Society for Gravitational and Space Research

Motivation for studying estrous cyclicity in simulated weightlessness

- STS-131, STS-133, STS-135 revealed cessation of estrous cycle in female mice (Tash 2012 & Ronca 2014)
- Spaceflight leads to loss of corpora lutea and significantly reduced estrogen receptor mRNA levels in the uterus

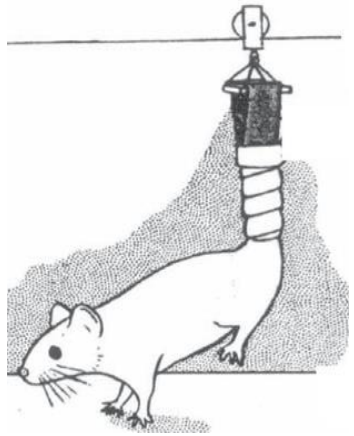
Goals of this study

- Assess whether female endocrine signaling biomarkers are altered in simulated weightlessness via hindlimb unloading model in both reproductive and non-reproductive organs



Ground **Flight**

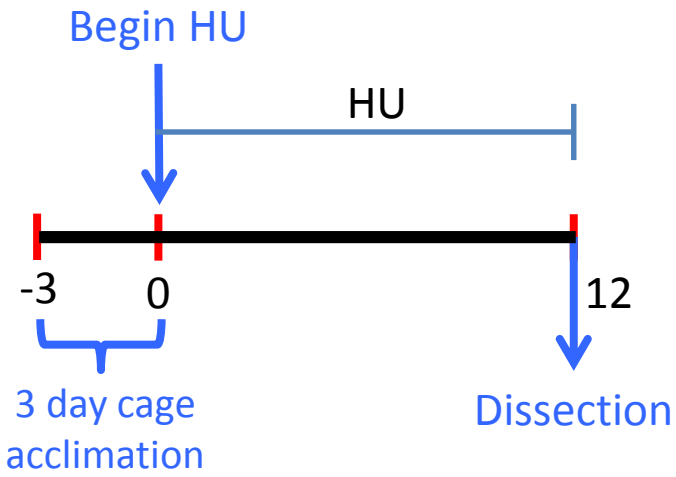
Unpublished images from Tash



Hindlimb Unloading (HU)
16 wk C57BL6 female mice

Experimental Design

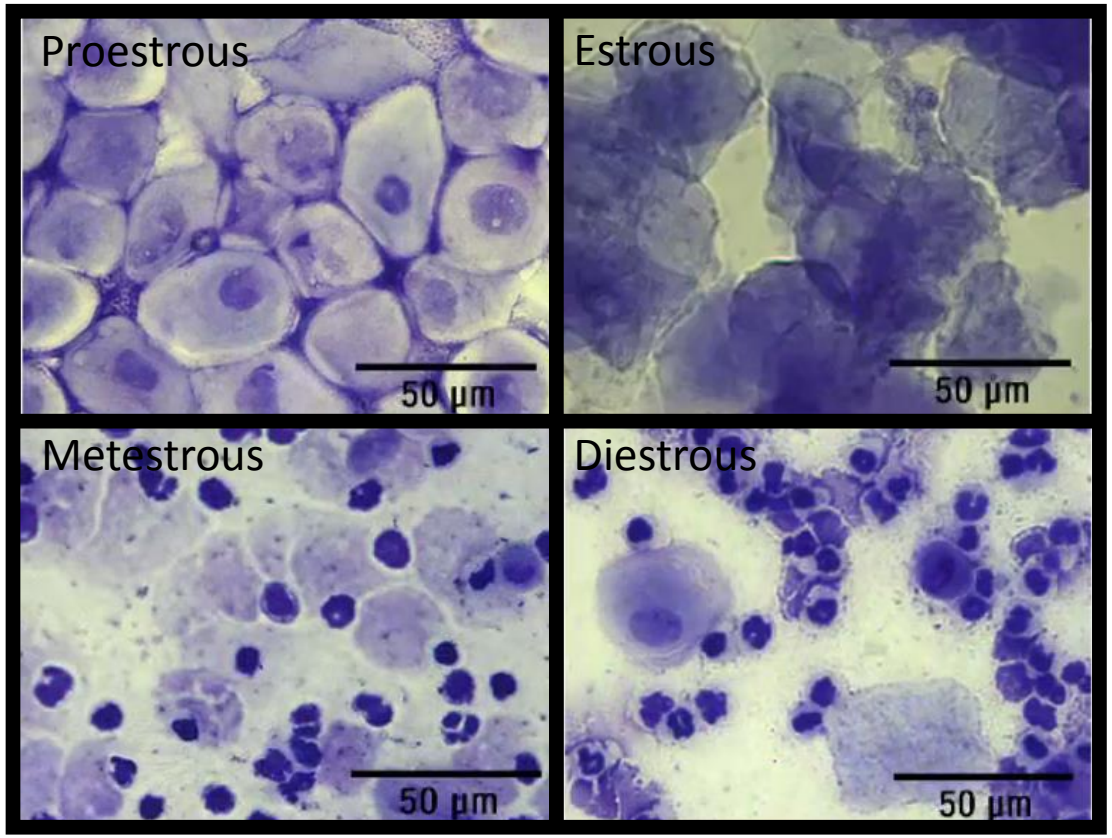
- Normally Loaded n=10
- Hindlimb Unloaded n=10
- Vivarium Control n=10



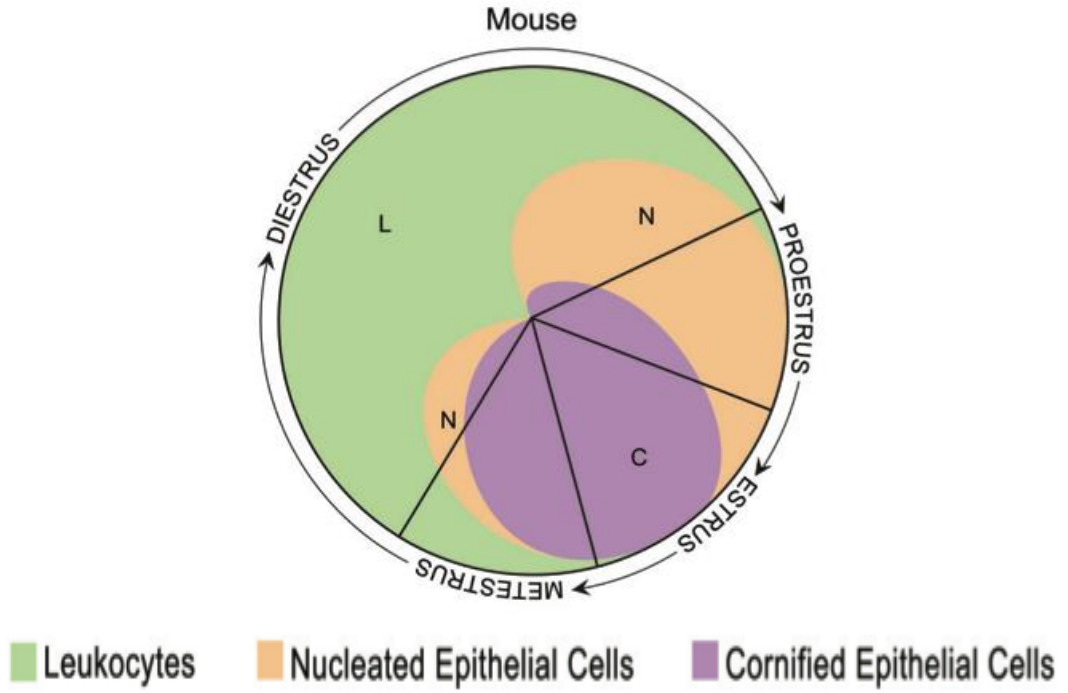
Primary endpoints:

1. Did mice maintain/return to normal estrous cycling?
2. Were there structural changes to reproductive organs (ovaries, uterus, vaginal wall)?

Methods: Daily lavage and Imaging



McLean, A. C., Valenzuela, N., Fai, S., Bennett, S. A. Performing Vaginal Lavage, Crystal Violet Staining, and Vaginal Cytological Evaluation for Mouse Estrous Cycle Staging Identification. *J. Vis. Exp.* (67), e4389, doi:10.3791/4389 (2012).



Cora, Michelle C., Linda Kooistra, and Greg Travlos. "Vaginal cytology of the laboratory rat and mouse: review and criteria for the staging of the estrous cycle using stained vaginal smears." *Toxicologic pathology* 43.6 (2015): 776-793.

Methods: Cytology Analysis

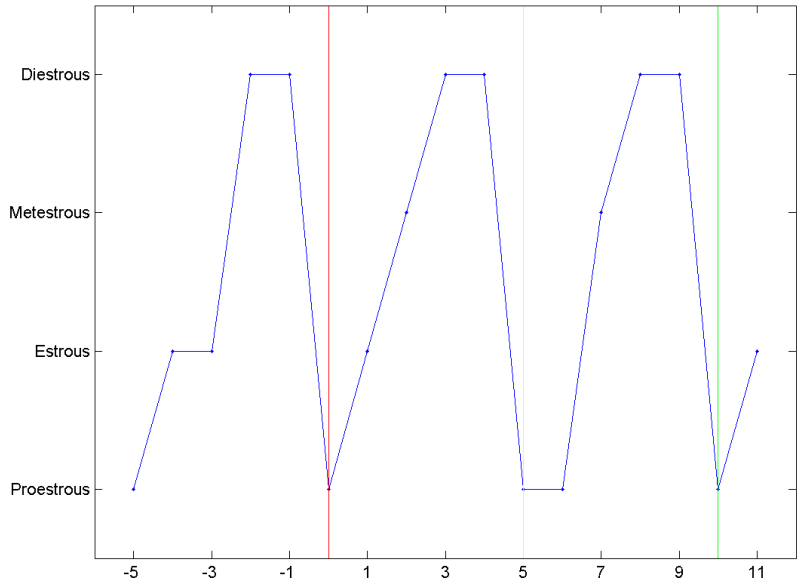
- Translate qualitative date into an experiment timeline for each mouse defining Day 0 as start of treatment.

- Graph each animal's estrous cycle in relation to other experiment landmarks

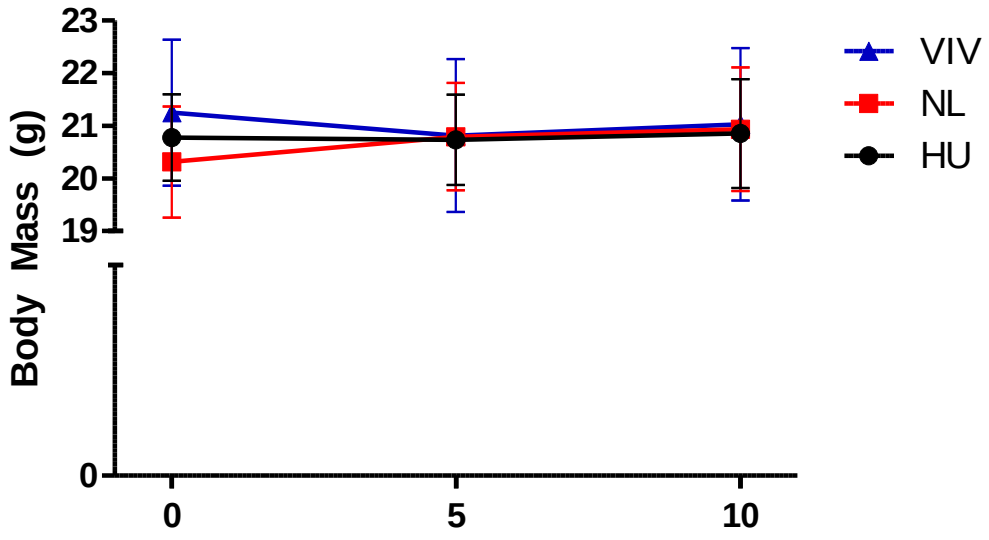
Hypothesis

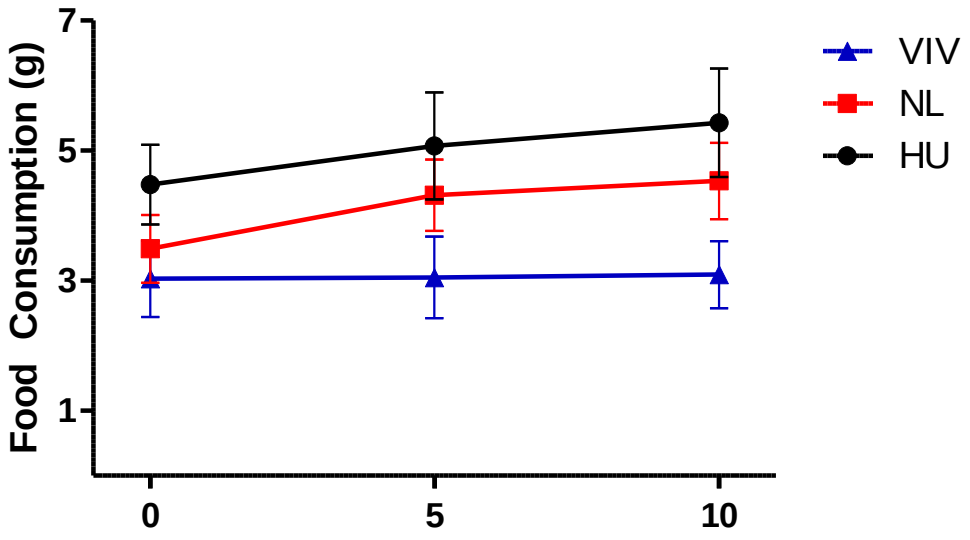
- Hindlimb unloading will cause mice to arrest estrous cyclicity in the diestrous stage

Experiment Landmark	Day
HU Cage Acclimation Begins	-3
HU Treatment Begins	0
Euthanasia/Tissue Collection	11/12

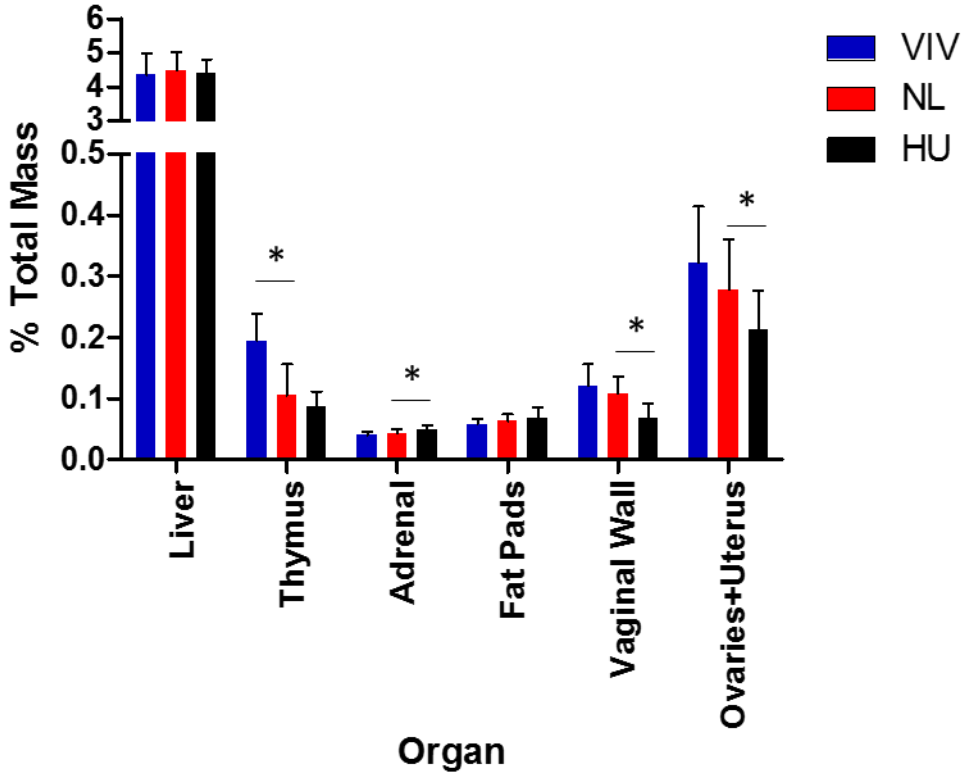


Result: Pair-feeding/Cage effect observed

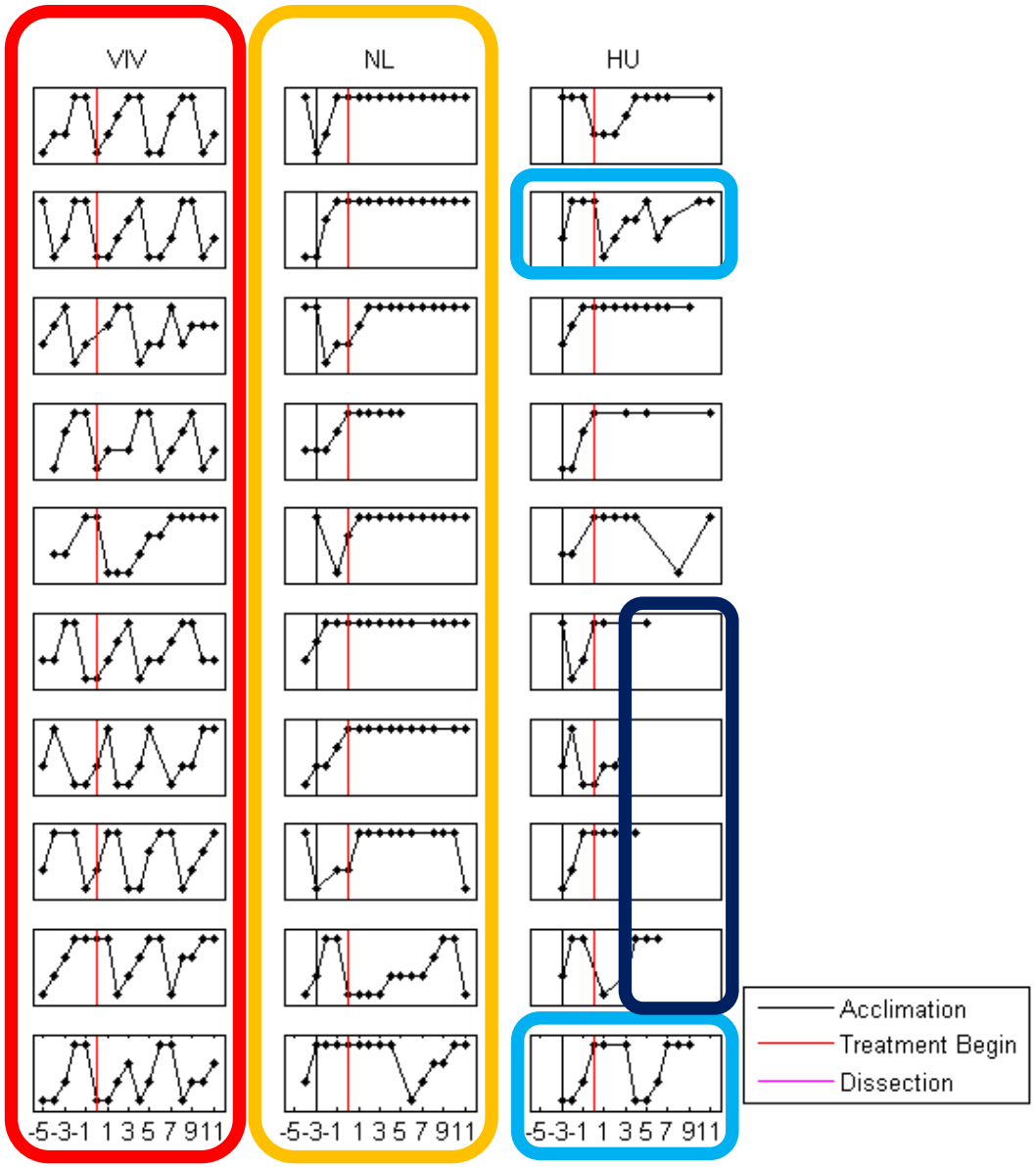




Result: Differences observed in HU reproductive organs and adrenals



Cytology Results



- Average cycle length of vivarium cage control animals was 4.7 days.
  - In line with reported literature[1,2]
  - Suggests our technique did not impede normal cycling
- Normal cycling did not present in many animals during the acclimation to HU cages, or even throughout remainder of experiment.
- Some occurrences of cycling did return to HU cage mice.
- Infection-like symptoms resulted in missing data in HU mice.

1. James F. Nelson, Lêda S. Felicio, Patrick K. Randall, Clifford Sims, Caleb E. Finch; A Longitudinal Study of Estrous Cyclicity in Aging C57BL/6J Mice: I. Cycle Frequency, Length and Vaginal Cytology. Biol Reprod 1982; 27 (2): 327-339. doi: 10.1095/biolreprod27.2.327
2. Byers SL, Wiles MV, Dunn SL, Taft RA (2012) Mouse Estrous Cycle Identification Tool and Images. PLoS ONE 7(4): e35538. https://doi.org/10.1371/journal.pone.0035538

Conclusions

- Cage effect/Pair Feeding effect present
- Validated model for observing estrous stage in VIV control
- Longer acclimation period may allow control cage animals to return to normal estrous cyclicity
- Longer HU period may allow HU animals to acclimate and return to normal estrous cyclicity

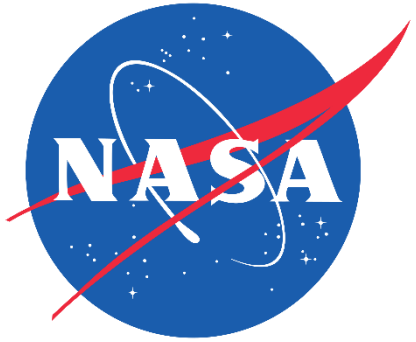
Thanks to those who contributed

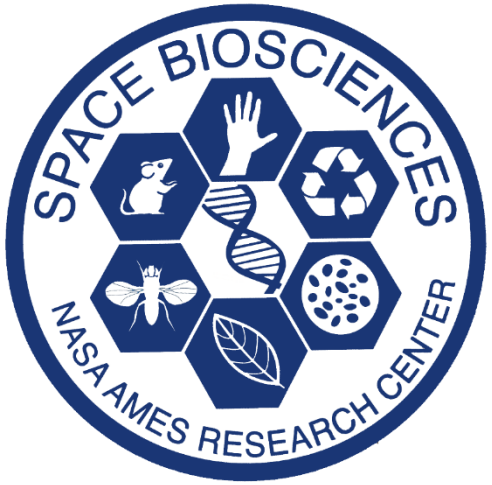
NASA Ames Research Center

- **Joshua Alwood**
- Catherine Choi
- Parker Dubee
- Ayana Kishibuchi
- Eric Moyer
- Kotaro Okada
- Megan Pendleton
- **April Ronca**
- Ryan Scott
- Pantelis Solomides
- Brad Staten
- Yuli Talyansky
- Nicholas Thomas

Kansas UMC

- **Lane Christenson**
- **Joseph Tash**





This research was supported by NASA Space Biology Grant NNX15AB48G